

## Do non-additive effects on decomposition in litter-mix experiments result from differences in resource quality between litters?

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Differences in resource quality between litter species have been postulated to explain why litter-mixtures may decompose at a different rate to that which would be predicted from single species litters (termed 'non-additive effects'). In particular, positive, non-additive effects of litter-mixing on decomposition have been explained by differences in initial nitrogen concentration between litter species. This interpretation is confounded because litter species that differ in nitrogen content also differ by a number of other resource quality attributes. Thus, to investigate whether initial nitrogen concentration does account for positive, non-additive effects of litter-mixing, we mixed grass litters that differed in initial nitrogen concentration but not species or structural plant part identity, and then followed mass loss from the litter-mixes over time. We used the litterbag technique and three grass species for which a gradient of four distinct initial nitrogen concentrations had been generated. We produced all no- to four-mix compositions of litter qualities for each species. Litter from different species was never mixed.

Contrary to what would be predicted, we found that when litters of the same species but with different initial nitrogen concentrations were mixed, that negative, non-additive effects on decomposition were generally observed. In addition, we found that once mixed, increasing litter quality richness from two to four mixtures had no significant, non-additive effect on decomposition. Litter quality composition explained little of the experimental variation when compared to litter quality richness, and different compositions generally behaved in the same manner. Our findings challenge the commonly held assumption that differences in nitrogen concentration between plant species are responsible for positive, non-additive effects of litter-mixing on decomposition.

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Plant litter decomposition plays a critical role in nutrient cycling and organic matter turnover within ecosystems, processes that are important determinants of plant productivity and ecosystem carbon storage (Petersen and Luxton 1982, Vitousek 1982, Aerts 1997). The most important factors controlling decomposition rate are climate, resource quality and soil organisms (Swift et al. 1979). Seastedt (1984) suggested that, due to differences in resource quality between species, litter-

mixtures might decompose at a different rate to that which would be predicted from single species litterbags. This suggestion has prompted experimental work to investigate the contribution of litter species richness and composition to decomposition rate (reviewed by Wardle et al. 1999). The work has been motivated by an interest in controls on litter decomposition in plant communities (Chapman et al. 1988, Blair et al. 1990, Salamanca et al. 1998), plant populations (Collins et al.

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1990, Quemada and Cabrera 1995), and by whether biodiversity is related to ecosystem functioning (Wardle et al. 1997, Hector et al. 2000). The 'litter-mix experiments' (Wardle et al. 1999) have shown: 1) positive, non-additive effects (i.e. mixture results are not simply the additive sum of single species results) on decomposition rate (Salamanca et al. 1998, Hector et al. 2000); 2) that decomposition is insensitive to changes in litter diversity (Blair et al. 1990, Rustad 1994, Snowcroft 1997, Knops et al. 2001); and 3) that strong, non-additive effects are apparent but highly idiosyncratic (Chapman et al. 1988, Fyles and Fyles 1993, Wardle et al. 1997). These different findings probably occur because when litter species are mixed a number of litter quality variables are varied simultaneously, some of which may promote positive, non-additive effects and others negative, non-additive effects (see below).

Litter quality can be defined in numerous ways (Swift et al. 1979, Melillo et al. 1982, Tian et al. 1992, Heal et al. 1997) and the measure that best predicts decomposition rate will vary between habitats and be dependent on the time course of the study (Taylor et al. 1989). For the early stages of decomposition, initial nitrogen concentration (or C:N ratio) of plant litter is generally a good predictor (Taylor et al. 1989, Hector et al. 2000). In litter-mix experiments where positive, non-additive effects on decomposition rate have been observed, differences in initial nitrogen concentration are postulated to account for the results (Wardle et al. 1997, Salamanca et al. 1998, Hector et al. 2000). The mechanisms responsible include translocation of nitrogen from high to low nitrogen-containing litters (Seastedt 1984, Chapman et al. 1988), possibly via fungal hyphae (McTiernen et al. 1997), facilitating more rapid decomposition of poorer quality litter, without a reduction in decomposition of the higher quality litter. In addition, high quality litter may support a larger soil organism community which 'spills over' onto the lower quality litter. Where decomposition has been shown to be either insensitive to litter-mixing or to respond in a negative, non-additive manner, the factors that negate positive effects or retard decomposition, respectively, include compounds or structures which inhibit decomposition, e.g. phenolics and hairs (Chapman et al. 1988, Hector et al. 2000). Thus litter species which differ in initial nitrogen concentration may also differ in a number of other attributes associated with resource quality, some of which may inhibit decomposition of mixed litters. To quantify the potential effect on decomposition rate of mixing litters with different initial nitrogen concentrations, these confounding attributes of resource quality must be controlled for.

Our aim was to determine whether positive, non-additive effects on decomposition in litter-mix experiments are the result of differences in initial nitrogen concentration between litters. We investigated this by mixing grass litters that differed in initial nitrogen

concentrations (termed 'litter quality' for brevity) but not species or structural plant part identity, and then by following mass loss from these litter-mixes over time. We hypothesised that decomposition rate would increase with higher litter quality richness due to nitrogen 'fertilisation' and soil organism 'spill-over' effects. We also hypothesised that, in the absence of confounding variables, that compositional effects would not be idiosyncratic.

## Methods

### Site

The experiment was conducted at Silwood Park, UK (51°22'N, 00°37'W) in a semi-natural acid grassland (soil pH 5.26 in 0.01M CaCl<sub>2</sub>) fenced since 1998 to protect against rabbit and deer. The site had an altitude of 50 m a.s.l and a mean annual rainfall of 652 mm. The soil was a sandy loam with a clay:silt:sand ratio of 10:8:82% w/w. Experimental plots were free of herbicide, pesticide and fertiliser application (see Hector et al. 2000 for a full description).

### Litter preparation

Monocultures of *Arrhenatherum elatius* L., *Holcus lanatus* L. and *Agrostis capillaris* L. were grown under controlled light, temperature and humidity in 25 cm Ø pots filled with nutrient poor, sandy soil. The three species were chosen as they are common in UK grasslands and were found in the experimental site. Twice a week, for ten weeks, each pot received 18.75 µl of modified Hoagland's solution in 1 l of water, ensuring complete control of nutrient supply to the plants (Cotrufo et al. 1995). To manipulate plant nitrogen content, NH<sub>4</sub>NO<sub>3</sub> in the solution was added at four different concentrations: 5.5, 55, 150 and 330 mg N l<sup>-1</sup>. This produced four distinct plant tissue qualities which, hereafter, will be referred to from lowest to highest quality as A, B, C and D, respectively (Table 1).

To ensure a strongly pronounced quality gradient, aboveground tissue was harvested green and then dried at 40°C. Total C and N content of the litter was determined by coupled combustion chromatography analysis (NRM Ltd, Bracknell, UK).

### Experimental design

Litterbags (9 × 8 cm) were constructed from nylon mesh (Northern Mesh, Oldham, UK) with a 2.8 mm pore; bag edges were heat-sealed. Individual bags were filled with 0.9–1.1 g of litter from one plant species only and the quality richness level was manipulated

Table 1. Initial nitrogen concentration (N) and carbon: nitrogen ratio (C:N) of the aboveground tissue of three grass species. Plants were watered with solutions containing different amounts of  $\text{NH}_4\text{NO}_3$  to produce a gradient of four distinct litter qualities (A–D). Mass loss values from litterbags after field exposure are shown. Loss data (mean + 1 SE) were calculated from arcsine square-root transformed data at the block level ( $n = 5$ ).

Species	Quality	N (% w/w)	C:N	Litter mass (% of original)	
				30 d	60 d
<i>Arrhenatherum elatius</i>	A	1.83	23:1	53.8+2.25	39.0+2.03
	B	2.24	20:1	50.1+2.93	38.1+1.58
	C	2.71	16:1	48.2+2.16	37.6+1.20
	D	3.45	13:1	45.2+1.32	40.1+1.81
<i>Holcus lanatus</i>	A	1.84	23:1	49.0+1.12	36.4+1.98
	B	1.95	22:1	44.9+0.20	36.3+2.21
	C	2.28	19:1	38.1+0.69	34.0+1.53
	D	2.80	15:1	33.8+1.08	33.3+1.14
<i>Agrostis capillaris</i>	A	1.91	22:1	43.2+1.56	27.0+2.80
	B	2.45	18:1	41.7+1.06	26.0+2.78
	C	3.04	15:1	42.6+0.99	26.0+3.57
	D	3.50	13:1	40.3+1.78	23.1+3.18

from one to four (Table 2). All possible compositions of the litter qualities A–D within a richness level were generated (Table 2). This experimental design ensured that the mean nitrogen content for each richness level, within a species, was the same because each richness level contained the same proportional amount of litters A, B, C and D.

Two litterbags per species of each quality composition were incorporated into the litter layer in each of five  $3 \times 3$  m blocks on 22 May 2001. One bag was retrieved after 30 and 60 days. In total, 450 litterbags were used. Dry ( $40^\circ\text{C}$ ) mass of remaining litter was determined after separation from foreign material (soil, shoots, roots, fauna). We refer to mass loss from the bags as “decomposition” but recognise that in the strictest sense, this mass loss is the result of both the physical process of “breakdown” and the catabolic process of “decomposition” (Anderson 1973, Bradford et al. 2002).

### Statistical analyses

To determine how litter quality richness and composition affected litter decomposition a full factorial analysis of variance, with quality composition nested within quality richness, was used. Time and species identity were included as interacting factors; block did not interact with any of the other model factors. Data were expressed as ‘proportion of litter mass remaining’ and

were arcsine square-root transformed to meet the assumptions of ANOVA. To investigate the highest order significant interactions, data were divided into sub-sets based on one of the factors from the interaction term (e.g. by time) and then subjected to ANOVA. At the point when no significant interaction terms were detected with ANOVA, compositions of the same richness level were grouped into a single mean replicated only at the block level ( $n = 5$ ) and then these richness level means were compared using sequential Bonferroni planned contrasts (Sokal and Rohlf 1995). Four planned contrasts presented themselves, given that our specific study objectives in relation to richness were: 1) that the action of mixing litters with different qualities would increase decomposition; and 2) that once mixed decomposition would increase as quality richness increased. The specific contrasts were: 1) mean of richness level 1 (monoculture bags) vs grand mean of richness levels 2–4 (mixed bags); and 2) mean comparisons of richness levels 2 vs 3, 2 vs 4 and 3 vs 4. Note that because all possible compositions were produced at each richness level, richness terms with  $P < 0.05$  indicate that there were significant non-additive effects of litter-mixing between at least two of the four richness levels. The contrasts permitted investigation of the specific richness levels that differed at  $P < 0.05$ .

Although the above analysis did permit investigation of whether composition had a significant effect on decomposition, it did not permit elucidation of whether decomposition rates of different litter compositions be-

Table 2. Litter quality combinations.

Richness level (No. of qualities combined within a litterbag)	Amount of each quality within a litterbag (%)	Quality combinations (composition)
1	100	A, B, C, D
2	50	AB, AC, AD, BC, BD, CD
3	33	ABC, ABD, ACD, BCD
4	25	ABCD

haved idiosyncratically. That is, whether the impact of mixing was, in non-additive terms, positive, negative or null for a specific litter quality composition (regardless of the mean response across compositions at a specific richness level). In order to test this, the observed mixture rates were compared with the values that would have been expected based on the monoculture litterbags for the component litter qualities if no interaction effects were present (Blair et al. 1990, Wardle et al. 1997, Bardgett and Shine 1999) and the data plotted. Calculations were performed separately for each replicate block. Following Wardle et al. (1997), the expected litter mass remaining ( $R_e$ ) for each mixture was calculated by considering all the component qualities as follows:

$$R_e = \sum_{i=1}^S M_i/S$$

where:  $M_i$  = value for monoculture of quality  $i$ ;  
 $S$  = total number of qualities in mixture.

## Results

Results of the full factorial analysis of variance to determine how litter quality richness and composition affected litter decomposition are shown in Table 3. The significant three-way interaction between quality richness, species identity and removal time ( $P < 0.01$ , Table 3) indicated that quality richness did have a significant non-additive effect on decomposition but that the way it affected decomposition was not consistent across time or across species. Thus, we investigated each removal time separately and found that the only significant interaction was between species identity and quality richness (Day 30:  $F_{6,22} = 9.8$ ,  $P < 0.001$ ; Day 60:  $F_{6,22} = 36$ ,  $P < 0.001$ ). This indicated that the relative

effect of quality richness on decomposition was different between at least two of the grass species, making it necessary to analyse the effect of quality richness for each species separately at each time point. Quality richness had a significant effect ( $P < 0.001$ ) on mass loss for each species at each removal time except for *Arrhenatherum elatius* after 60 days of exposure ( $P > 0.05$ ). We investigated the significant quality richness terms to establish which richness level means differed within a species and removal time.

The analyses showed that the action of mixing litters produced negative, non-additive effects on decomposition. That is, in every case where quality richness was a significant factor in ANOVA, mass loss from mixture litterbags was significantly less ( $P < 0.001$ ) than from monoculture bags (Fig. 1). In contrast, an increase in richness once litters were mixed had no significant impact ( $P > 0.05$ ) on decomposition (Fig. 1).

We plotted observed minus expected litter mass remaining data (Fig. 2) to investigate whether specific litter quality compositions behaved similarly or idiosyncratically. For all mixture compositions of *H. lanatus* and *Agrostis capillaris*, at both removal times, observed values were higher than expected values (Fig. 2). The same pattern was observed for *Arrhenatherum elatius* at 30 days. However, at 60 days for *A. elatius*, whether observed values were higher than, lower than or the same as expected values was dependent on a specific mixture composition (Fig. 2).

Due to the fact that the richness level comparisons using the proportion mass remaining data were made for each species per time point, we could use regression analyses on the observed minus expected values of mass loss to confirm/question the statistical results of the planned contrasts. The regression approach has the advantage of higher degrees of freedom because compositions were not grouped to produce individual means per richness level. Regression slopes for litter

Table 3. Summary of the analyses of the litter quality diversity experiment. Data (proportion of litter mass remaining) were arcsine square-root transformed prior to analysis. Significant effects ( $P < 0.05$ ) are shown in bold. Quality richness terms (indented) are tested against the quality composition term shown in the row immediately beneath them; all other terms are tested against the overall model residual.

Source of variation	d.f.	s.s.	% s.s.	m.s.	F	P
Removal time (Rt)	1	17 023.1	50.7	17 023.1	<b>2250.14</b>	<b><math>2.2 \times 10^{-16}</math></b>
Species identity (Si)	2	538.7	1.6	269.4	<b>35.61</b>	<b><math>8.4 \times 10^{-15}</math></b>
Block	4	62.2	0.2	15.5	2.06	0.0863
Quality richness (Qr)	3	8465.7	25.2	2821.9	<b>110.23</b>	<b><math>1.7 \times 10^{-8}</math></b>
Quality composition (Qc)	11	281.6	0.8	25.6	<b>3.38</b>	<b>0.0002</b>
Rt × Si	2	245.4	0.7	122.7	<b>16.22</b>	<b><math>1.8 \times 10^{-7}</math></b>
Rt × Qr	3	1367.4	4.1	455.8	<b>49.54</b>	<b><math>1.1 \times 10^{-6}</math></b>
Rt × Qc	11	101.2	0.3	9.2	1.22	0.2745
Si × Qr	6	2206.4	6.6	367.7	<b>31.43</b>	<b><math>1.0 \times 10^{-9}</math></b>
Si × Qc	22	256.3	0.8	11.7	1.54	0.0584
Rt × Si × Qr	6	215.2	0.6	35.9	<b>5.06</b>	<b>0.0021</b>
Rt × Si × Qc	22	156.2	0.5	7.1	0.94	0.5441
Residual	349	2640.3	7.9	7.6		
Total	442	33 560	100	75.9		

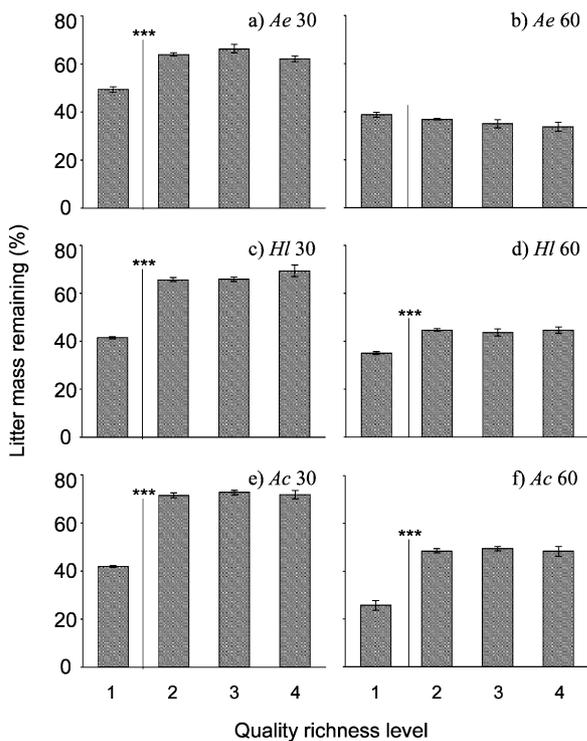


Fig. 1. Effect of litter quality richness on mass loss of a,b) *Arrhenatherum elatius*, c,d) *Holcus lanatus*, e,f) *Agrostis capillaris* litter after 30 (a,c,e) and 60 (b,d,f) days of field exposure. Mass remaining (mean  $\pm$  1 SE; percentage of original) in litterbags is shown. Means and standard errors were calculated from arcsine square-root transformed data at the block level ( $n = 5$ ) and then back transformed. Richness level 1 means (i.e. no litter mixing) that are significantly different to the grand mean of richness levels 2–4 (i.e. mixed litter), within a species and removal time, are marked as: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . These markings are also used to show differences between the means for richness levels 2–4.

mass remaining versus richness level were not significant ( $P > 0.05$ ; Fig. 2), confirming that richness level (from 2–4) was not a significant determinant of mass loss. However, the grand mean across all litter quality compositions of the observed minus expected litter mass remaining was significantly different in every case (Fig. 2; determined by testing the average deviation across all litter mixtures vs the null expectation of zero). While confirming the significant differences between monoculture and mixed bags detected using planned contrasts, this also indicated that the mass loss from mixed *A. elatius* litter at 60 days was significantly greater ( $P < 0.01$ ) than from the monoculture bags, whereas it was shown to be non-significant using the planned contrasts.

## Discussion

Differences in initial nitrogen concentration between litter species are hypothesised to account for positive,

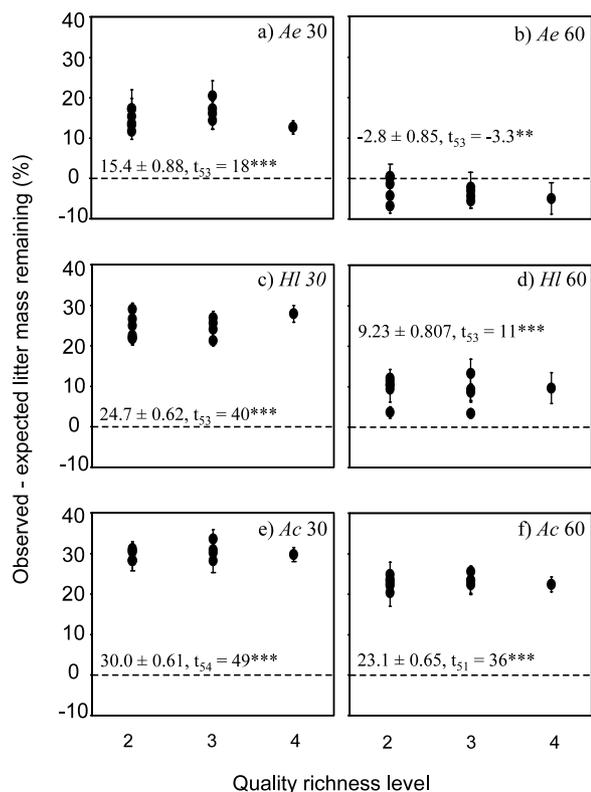


Fig. 2. Deviations from the expected effect (based on monocultures) of litter quality diversity on mass loss of a,b) *Arrhenatherum elatius*, c,d) *Holcus lanatus*, e,f) *Agrostis capillaris* litter after 30 (a,c,e) and 60 (b,d,f) days of field exposure. Differences between observed and expected values for litter mass remaining (mean  $\pm$  1 SE; percentage of original) in litterbags are shown. Means and standard errors were calculated from arcsine square-root transformed data at the plot level ( $n = 5$ ). Each point represents a single litter quality composition and values greater than zero indicate a lower loss of litter than predicted from monocultures. Regression slopes for litter mass remaining versus richness level, within a species and removal time, are not shown because they were not significant ( $P > 0.05$ ). The grand mean  $\pm$  1 SE is shown on each graph with the t statistic and significance level: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

non-additive effects on decomposition observed in litter-mix experiments (Wardle et al. 1997, Hector et al. 2000). The certainty of this hypothesis is questionable given that litter species that vary in nitrogen concentration also vary in a number of other traits. These 'other traits' may contribute to the positive, non-additive effects, solely account for them, cancel them out or, even, produce negative, non-additive effects. Hence, idiosyncratic effects of litter-mixing on decomposition are common (Kaneko and Salamanca 1999, Wardle et al. 1999, Hector et al. 2000) and any mechanistic interpretations are ultimately confounded. Our experiment was designed to permit investigation of the effect of litter nitrogen concentration diversity, in isolation of confounding variables associated with mixed-species litters,

on decomposition. Contrary to what would be predicted (Seastedt 1984, Wardle et al. 1997, Hector et al. 2000), we found that when litters of the same species but with different initial nitrogen concentrations were mixed, that negative, non-additive effects on decomposition were generally observed. In addition, we found that once mixed, increasing litter quality richness from two to four mixtures had no significant, non-additive effect on decomposition.

We believe that our experiment is the first litter-mix experiment that set out to test whether diversity of a single factor, in the absence of confounding variables, causes non-additive effects on litter decomposition. Other investigators (Collins et al. 1990, Quemada and Cabrera 1995) have varied litter quality diversity within single-species litterbags. They found positive, non-additive effects on decomposition rate (measured as CO<sub>2</sub> evolved) of mixing cereal crop residues (stems, leaves, etc) during the first 30 days of incubation. These residues differed markedly by a number of quality variables (including nitrogen concentration) and, so, as in litter species mix-experiments, it is not possible to categorically state which quality variable(s) was responsible for the observed non-additive effects. We used homogenised, vegetative, foliar material to overcome this. It could be argued that our method of preparing litters with different nitrogen concentrations caused other quality-related variables, such as lignin content, to vary. We did not assess this but findings from other litter-mix experiments (Wardle et al. 1997) do support our observation that mixing grass litters of different nitrogen concentrations causes negative, non-additive effects on decomposition.

Wardle et al. (1997) found that decomposition from mixed-grass species litterbags, removed from the field after 20 days, decreased in a non-additive manner when diversity was increased from one to two species. In addition, decomposition was not markedly affected when grass species richness was increased from two across to eight and all litter-mix compositions behaved in the same way (i.e. non-additive decrease in decomposition) at this 20 day point. We observed a similar pattern for our 30 day removal point (Fig. 2a,c,e), with a non-additive, and consistent, decrease in decomposition due to mixing but no effect of richness level (2–4). After 60 days of field exposure, the impact (positive, negative or null) of diversity on decomposition from our bags was dependent on the mixture investigated (Fig. 2b,d,f) and a similar pattern was observed by Wardle et al. (1997) for their second removal point (80 days). Wardle et al. concluded that litter-mixing can have large but not easily predictable impacts on litter decomposition: this conclusion was based on litter-mixes of species within and between four functional groups (cropping and grassland dicotyledonous herbs, grasses, trees). However, our findings and those of Wardle et al. (1997) suggest that in the early stages (up

to 30 days) of grass decomposition that large and predictable (negative, non-additive) impacts of litter-mixing on decomposition will occur. The two experiments (Wardle et al. 1997, current study) support the hypothesis that negative, non-additive effects on decomposition are produced by mixing grass litters (of the same or different species) with different initial nitrogen concentrations. Whether this hypothesis holds for other plant functional types can only be answered by studies such as the current one, in which investigators set out to vary only one litter quality variable in order to remove the influence of confounding variables.

Why would the mixing of different litter qualities retard decomposition? One potential mechanism is through an impact on decomposer organisms. Blair et al. (1990) found that the abundance of fungi and bacteria in mixed species litterbags (the species varied in litter quality) was either similar or lower than that in single species litterbags. Microarthropod abundance was more variable but also tended to be lower than predicted. If mixing of litter qualities in our study brought about a reduction in microflora and fauna then this could explain the reduced decomposition rate in mixed bags. In support of this contention, Bardgett and Shine (1999) reported that intermediate richness levels (between 2–4) reduced microbial biomass, although Wardle et al. (1997) observed that microbial biomass varied idiosyncratically across different grass-litter species mixes.

The suggestion that mixing litters of different initial nitrogen concentrations might non-additively reduce microbial and faunal populations does not, in the same way that our results do not, support hypotheses that nitrogen concentration diversity produces positive, non-additive effects on decomposition. What evidence is there that initial nitrogen concentration was the factor responsible for the negative, non-additive effects of mixing we observed? Inspection of mass loss from our monoculture bags (Table 1) revealed that there was only a significant ( $P < 0.01$ ) positive relationship between litter loss and nitrogen concentration for *A. elatius* and *H. lanatus* at 30 days. For *A. capillaris* at 30 days, and for all species at 60 days, no relationship was observed ( $P > 0.05$ ; Table 1). Yet, at 30 days mass loss from all species responded in an identical manner when litter was mixed (Fig. 1a,c,e, 2a,c,e) and at 60 days the response of decomposition to litter-mixing depended on species identity (Fig. 2b,d,f). Therefore, the impact of nitrogen concentration on mass loss from monoculture litterbags was seemingly unrelated to the effects we observed when litters were mixed. This does not in itself rule out the possibility that differences in initial nitrogen concentration between litters were responsible for the non-additive effects we observed (especially as we attempted to vary only this single factor) but it does not support it.

Further evidence that initial nitrogen concentration may not have been responsible for the effects we observed comes from examination of the results of the full-factorial analysis of variance. Quality richness, as a single factor in the analysis of the litter quality diversity experiment, accounted for 25% of the sums of squares, whereas quality composition accounted for only 0.8% (Table 3). Thus, simply mixing litters explained a much greater proportion of our treatment differences than did combining litters differing in nitrogen concentration. Based on these arguments we propose that the negative, non-additive impacts we observed resulted from spatial variability of leaf blades of different qualities within litterbags, rather than through differences in nitrogen concentration per se. That is, as soon as two (or more) different litters are mixed the litterbag becomes a markedly more heterogeneous nutrient patch than when only single litter types are used. We suggest that this marked heterogeneity precludes establishment of a subset of the soil community that is best adapted to decompose a particular litter quality and, as a result, faunal and microbial biomass is reduced in mixed bags, as observed by Blair et al. (1990) and Bardgett and Shine (1999).

At the 60 day removal point the non-additive effects of litter mixing, whether positive or negative, were less pronounced across all species than at 30 days (Fig. 2). From our study it is not possible to state which mechanism, or mechanisms, were responsible for this decrease in non-additive effects over time. However, a number of mechanisms may have contributed. First, differences in quality between litters may have become less pronounced over time, reducing the heterogeneity in a litterbag and thus the inhibition of microbial and faunal biomass we proposed to account for the negative, non-additive effects at 30 days. Second, hypothesised positive effects of mixing litters with different initial nitrogen concentrations (Seastedt 1984, Chapman et al. 1988, Blair et al. 1990, Wardle and Lavelle 1997) began to establish and counteract negative effects of mixing. Third, the litters began to switch to lignin-controlled decomposition (Berg 1986, Taylor et al. 1989): we did not intentionally vary lignin concentration and so would not expect to have observed lignin concentration diversity effects. The possibility of a shift to lignin-controlled decomposition raises an important point with regard to controls on litter decomposition. Initial N concentration is not a good predictor of decomposition rate for litter from all species (Melillo et al. 1982, Taylor et al. 1989) and carbon availability, not nitrogen, including the form of available carbon (Robinson et al. 1994), typically limits soil microbes (Aldén et al. 2001). Therefore, non-additive effects are unlikely to be simply explained by single litter quality parameters given that controls on decomposition may change with time, litter species and habitat (see also Wardle et al. 1997).

In conclusion, we found no evidence to support the hypothesis that differences in initial nitrogen concentration between litters can cause positive, non-additive effects when litters are mixed. Instead, we demonstrated that when litters of the same species but with different initial nitrogen concentrations were mixed, that negative, non-additive effects on decomposition were generally apparent. Longer-term studies using senesced litter, which attempt to control single quality variables, are required to further investigate the impacts of litter quality diversity on decomposition.

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## References

- Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. – *Oikos* 79: 439–449.
- Aldén, L., Demoling, F. and Bååth, E. 2001. Rapid method of determining factors limiting bacterial growth in soil. – *Appl. Environ. Microb.* 67: 1830–1838.
- Anderson, J. M. 1973. The breakdown and decomposition of sweet chestnut (*Castanea sativa* Mill.) and beech (*Fagus sylvatica* L.) leaf litter in two deciduous woodland soils. – *Oecologia* 12: 251–274.
- Bardgett, R. D. and Shine, A. 1999. Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. – *Soil Biol. Biochem.* 31: 317–321.
- Berg, B. 1986. Nutrient release from litter and humus in coniferous forest soils. A mini review. – *Scand. J. For. Res.* 1: 317–322.
- Blair, J. M., Parmelee, R. W. and Beare, M. H. 1990. Decay rates, nitrogen fluxes, and decomposer communities of single- and mixed-species foliar litter. – *Ecology* 71: 1976–1985.
- Bradford, M. A., Tordoff, G. M., Eggers, T. et al. 2002. Microbiota, fauna, and mesh size interactions in litter decomposition. – *Oikos* 99: 317–323.
- Chapman, K., Whittaker, J. B. and Heal, O. W. 1988. Metabolic and faunal activity in litters of tree mixtures compared with pure stands. – *Agric. Ecosyst. Environ.* 24: 33–40.
- Collins, H. P., Elliott, L. F., Rickman, R. W. et al. 1990. Decomposition and interactions among wheat residue components. – *Soil Sci. Soc. Am. J.* 54: 780–785.
- Cotrufo, M. F., Ineson, P. and Roberts, J. D. 1995. Decomposition of birch leaf litters with varying C-to-N ratios. – *Soil Biol. Biochem.* 27: 1219–1221.
- Fyles, J. W. and Fyles, I. H. 1993. Interaction of Douglas fir with red alder and salal foliage litter during decomposition. – *Can. J. For. Res.* 23: 358–361.
- Heal, O. W., Swift, M. J. and Anderson, J. A. 1997. Plant litter quality and decomposition: an historical overview. – In: Cadish, G. and Giller, K. E. (eds), *Driven by nature: plant litter quality and decomposition*. CAB International, pp. 3–30.
- Hector, A., Beale, A. J., Minns, A. et al. 2000. Consequences of the reduction of plant diversity for litter decomposition: effects through litter quality and microenvironment. – *Oikos* 90: 357–371.

- Kaneko, N. and Salamanca, E. F. 1999. Mixed leaf litter effects on decomposition rates and soil microarthropod communities in an oak-pine stand in Japan. – *Ecol. Res.* 14: 131–138.
- Knops, J. M. H., Wedin, D. and Tilman, D. 2001. Biodiversity and decomposition in experimental grassland ecosystems. – *Oecologia*. 126: 429–433.
- McTiernen, K. B., Ineson, P. and Coward, P. A. 1997. Respiration and nutrient release from tree litter mixtures. – *Oikos* 78: 527–538.
- Melillo, J. M., Aber, J. D. and Muratore, J. F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. – *Ecology* 63: 621–626.
- Petersen, H. and Luxton, M. 1982. A comparative analysis of soil fauna populations and their role in decomposition processes. – *Oikos* 39: 287–388.
- Quemada, M. and Cabrera, M. L. 1995. Carbon and nitrogen mineralized from leaves and stems of four cover crops. – *Soil Sci. Soc. Am. J.* 59: 471–477.
- Robinson, C. H., Dighton, J., Frankland, J. C. and Roberts, J. D. 1994. Fungal communities on decaying wheat straw of different resource qualities. – *Soil Biol. Biochem.* 26: 1053–1058.
- Rustad, L. 1994. Element dynamics along a decay continuum in a red spruce ecosystem in Maine. – *Ecology* 75: 867–879.
- Salamanca, E. F., Kaneko, N. and Katagiri, S. 1998. Effects of leaf litter mixtures on the decomposition of *Quercus serrata* and *Pinus densiflora* using field and laboratory microcosm methods. – *Ecol. Eng.* 10: 53–73.
- Seastedt, T. R. 1984. The role of microarthropods in decomposition and mineralization processes. – *Annu. Rev. Entomol.* 29: 25–46.
- Snowcroft, P. G. 1997. Mass and nutrient dynamics of decaying litter from *Passiflora mollissima* and selected native species in Hawaiian montane rain forest. – *J. Trop. Ecol.* 13: 407–426.
- Sokal, R. R. and Rohlf, F. J. 1995. *Biometry*, third edition. – W. H. Freeman and Co.
- Swift, M. J., Heal, O. W. and Anderson, J. M. 1979. Decomposition in terrestrial ecosystems. *Studies in Ecology*. Vol. 5. – Blackwell.
- Taylor, B. R., Parkinson, D. and Parsons, W. F. J. 1989. Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. – *Ecology* 70: 97–104.
- Tian, G., Kang, B. T. and Brussaard, L. 1992. Biological effects of plant residues with contrasting chemical compositions under humid tropical conditions: decomposition and nutrient release. – *Soil Biol. Biochem.* 24: 1051–1060.
- Vitousek, P. M. 1982. Nutrient cycling and nutrient use efficiency. – *Am. Nat.* 119: 553–572.
- Wardle, D. A. and Lavelle, P. 1997. Linkages between soil biota, plant litter quality and decomposition. – In: Cadish, G. and Giller, K. E. (eds), *Driven by nature: plant litter quality and decomposition*. CAB International, pp. 107–123.
- Wardle, D. A., Bonner, K. I. and Nicholson, K. S. 1997. Biodiversity and plant litter: experimental evidence which does not support the view that enhanced species richness improves ecosystem function. – *Oikos* 79: 247–258.
- Wardle, D. A., Giller, K. E. and Barker, G. M. 1999. The regulation and functional significance of soil biodiversity in agroecosystems. – In: Wood, D. and Lenné, J. M. (eds), *Agrobiodiversity: characterization, utilization and management*. CAB International, pp. 87–121.